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Research paper

Differences in transcript abundance of genes on BTA15 located within a region associated with gain in beef steers☆

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ABSTRACT

Using results from a previous GWAS, we chose to evaluate seven genes located within a 229 Kb region on BTA15 for variation in RNA transcript abundance in a library of tissue samples that included adipose, liver, rumen papillae, spleen, muscle, and small intestine epithelial layers from the duodenum, ileum and jejunum collected from steers (n = 14) with positive and negative residual GN near mean dry matter intake (DMI). The genes evaluated were two olfactory receptor-like genes (*LOC525033* and *LOC618173*), *RRM1*, *STIM1*, *RHOG*, *PGAP2*, and *NUP98*. The rumen papillae transcript abundance of *RHOG* was positively correlated with residual GN ($P = 0.02$) and ruminal *STIM1* exhibited a trend towards an association with residual GN ($P = 0.08$). The transcript abundance of one olfactory receptor (*LOC618173*) in the ileum was also positively associated with residual GN ($P = 0.02$) and *PGAP2* and *LOC525033* in the ileum displayed trends for association with GN ($P \leq 0.1$). To further evaluate the differential expression detected in the ileum and rumen of these animals, the transcript abundance of *STIM1* and *RHOG* in the rumen and of *PGAP2* and the olfactory receptors in the ileum were assessed in an additional group of 32 animals with divergent average daily gain (ADG) and average daily feed intake (ADFI) collected over two groups. The olfactory receptor, *LOC525033*, was not expressed in the ileum for the majority of these animals. Only *RHOG* showed a slight, but non-significant trend towards greater expression in animals with greater gain. We have detected differences in the transcript abundance of genes within this region in the rumen and ileum of animals selected for greater and less residual gain; however, we were unable to validate the expression of these genes in the larger group of cattle possibly due to the differences in phenotype or contemporary group.

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1. Introduction

Body weight gain is an important economic trait for both consumers and producers of feedlot steers. The ability of the animal to GN effectively and produce ample meat product during the time spent in the feedlot increases the producers profit as well as the yield of food for consumers. Gain is routinely measured within the population at U.S. Meat Animal Research Center (USMARC). Body weight gain (GN) over a 140 day feedlot study was acquired on over 1000 crossbred animals and used to analyze high density genotypic data from previously published genome wide association study (GWAS; Snelling et al., 2011).

Genetic markers with minor allele frequencies of 0.17–0.47 located between 51.79 and 52.03 Mb on BTA15 on the UMD3.1 genome assembly were associated with GN in the USMARC population of beef steers (nominal $P \leq 0.002$; Snelling et al., 2011). Four of the six SNP associated with GN in this region were in the top 100 SNPs on the Bovine SNP50 beadchip associated with GN. This region, and an additional 150 Kb of flanking sequence on each side of these markers, contains a cluster of 18 genes representing a number of olfactory receptor genes and potential RNA polymerase II subunit A C-terminal domain phosphatase pseudogenes. In addition to these genes, the genes *ribonucleotide reductase M1* (*RRM1*), *stromal interaction molecule 1* (*STIM1*), *ras homolog gene family, member G* (*RHOG*), *post-GPI attachment to proteins 2* (*PGAP2*) and *nucleoporin* 98 kDa (*NUP98*) also reside within this region. One of the associated SNP was located within an olfactory receptor gene (LOC618173), one was located within the *RRM1* loci and four were located within the *STIM1* gene loci. Of these SNP, only the SNP located within the *olfactory receptor, family 52, subfamily B, member 2-like*

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; BTA15, Bos Taurus Autosome 15; BW, body weight; DM, dry matter; DMI, dry matter intake; DNA, deoxyribonucleic acid; GN, gain; GWAS, genome wide association study; *LCORL*, ligand dependent nuclear receptor corepressor-like; M-MLV, moloney murine leukemia virus; *NUP98*, nucleoporin 98 kDa; PCR, polymerase chain reaction; *PGAP2*, post-GPI attachment to proteins 2; *RHOG*, ras homolog gene family, member G; RNA, ribonucleic acid; *RRM1*, ribonucleotide reductase M1; SNP, single nucleotide polymorphism; *STIM1*, stromal interaction molecule 1.

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gene (LOC618173) was within the coding region, changing a tyrosine at amino acid position 328 to an aspartic acid. The estimated effects of these SNP on GN were between ±4.5–4.8 kg over 140 days. Other GWAS studies have identified associations between SNP close to this region and cattle GN traits. They include body weight at weaning and weaning weight attributable to maternal milk (McClure et al., 2010). This same study also reported QTL for mature body weight and mature height slightly further downstream at 53 Mb on the UMD 3.1 genome assembly. In addition, several other SNPs in a GWAS at BTA15:42.7–42.8 Mb were associated with birth weight in a study by Lu et al. (2013). Thus there is additional evidence that a gene(s) in this region contributes to GN in cattle.

Some of the known functions of the genes residing between BTA15: 51.8–52.2 Mb are as follows: the RRM1 protein is a subunit of the ribonucleotide reductase complex which produces deoxyribonucleotides for DNA synthesis (Valsecchi et al., 2012). STIM1 is a transmembrane protein that mediates the influx of calcium after intracellular stores have been depleted. The *RHOG* gene encodes a rho family small GTPase that functions in signal transduction. Human mutations in the *PGAP2* gene cause the Mabry Syndrome which can be severe with intellectual disability and distinct facial features (Krawitz et al., 2013; Hansen et al., 2013); however, other symptoms can include shortening of bones and some digestive tract abnormalities. Nucleoporin 98 kDa (NUP98) is one of many proteins that make up the nuclear pore complex for transport of molecules in and out of the nucleus. There is limited information regarding the involvement of these genes in growth or GN in humans or animals. Thus, we chose to examine the transcript abundance of these five gene products and two olfactory receptors within this chromosomal region over a panel of tissues from 14 steers with positive and negative residual GN to determine whether we could associate the abundance of these genes with GN in cattle.

Because no specific relationship between feed intake or gain with these genes has been previously established, we chose to examine their expression in a panel of tissues that are likely to influence cattle feed efficiency. The panel of tissues included rumen, small intestine, liver, muscle, adipose and spleen. It is thought that efficient animals have lower maintenance energy costs (Richardson and Herd, 2004). The rumen and small intestine are large organs that have significant maintenance energy requirements, and differences in their ability to process and absorb nutrients may affect feed efficiency. The liver, while only 1.2% of the animal's body weight consumes 25% of the animal's energy, thus differences in liver function could play a role in feed efficiency. A previous study by Mader et al. (2009) illustrated a trend between the size of the liver and ADG ($P = 0.06$). This study also presented a correlation between spleen weight and G:F, a measure of feed efficiency. The spleen has an important role in immune function as the largest lymph node in the body and CNV of genes involved in immune function have been associated with high and low residual feed intake in cattle (Hou et al., 2012). Subcutaneous adipose tissue was included in the panel due to its likely role in cattle feed efficiency. Weight of subcutaneous fat was correlated to the G:F ration in feed lot steers (Mader et al., 2009). Moreover, previous studies suggest that efficient animals have an increase in lean tissue and a decrease in fat (Arthur et al., 2001; Richardson et al., 2001; Basarab et al., 2003), thus longissimus dorsi was also included in the panel of tissues for evaluation. A final goal of this study was to identify gene expression differences in cattle that would be robust across breed, thus we tested these genes for associations with GN on discovery and validation populations of animals from crossbred populations that included many breeds of cattle.

2. Methods

2.1. Animal care and use

All animal procedures were reviewed and approved by the USMARC Animal Care and Use Committee. Procedures for handling cattle complied with those specified in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

2.2. Animal populations

Discovery Population (Animals with positive and negative residual gain, n = 14 from 142 animals on study): Feed intake and growth were measured on fall-born steers (n = 142) that were part of a breed evaluation study (Kuehn et al., 2008). Breeds represented in this group included Angus, Beefmaster, Brangus, Brahman, Braunvieh, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, Red Angus, Salers, Santa Gertrudis, Shorthorn, and Simmental. Sires and maternal grand-sires were influential industry bulls for each breed. At the start of the study, steers were 348 ± 35 days of age and weighed 444 ± 4 kg. Feed intake and growth were evaluated for a 70-day period. Steers had *ad libitum* access to a diet that as a percentage of dry matter (DM) contained the ingredients listed in Table 1. Feed intake was measured using an Insentec Roughage Intake Control Feeding System (Insentec B.V., Marknesse, The Netherlands), and total DMI was summed over the feeding period. Steers were weighed on 0, 1, 21, 42, 56, 69, and 70 days of study. Body weight was quadratically regressed on days of study for each steer, and total BW GN was calculated from the regression equations. Total BW GN was regressed on total DMI. Seven steers with positive residual gain and seven steers with negative residual GN whose DMI was within 0.32 standard deviation of the mean intake were selected for subsequent measurements. Steers remained on the same diet following the end of the feed intake and growth study. Steers were slaughtered 12 to 21 days after the feed intake and growth study, and tissues were collected and immediately frozen in liquid nitrogen. The total breed composition of the seven crossbred animals from the high residual GN group was: 21% Angus, 21% Charolais, 7% Chiangus, 7% Hereford, 7% MarcII, 18% Salers, 4% Santa Gertrudis, and 14% Simmental. The breed composition of the low residual gain group was: 14% Angus, 14% Brown Swiss, 7% Charolais, 11% Gelbveih, 7% Hereford, 4% MarcI, 7% MarcII, 7% Maine Anjou, 7% Salers, 4% Shorthorn and 18% Simmental.

Validation Population (Divergent ADG and ADFI animals, n = 32 from a total of 327 animals on study): Steers born in the spring (n = 188) and fall of 2011 (n = 139) were evaluated individually for feed intake with an Insentec system over 64- and 92-day periods, respectively. The age of steers at the beginning of these trials was 344 ± 48 days. Steers were given *ad libitum* access to rations (DM basis) as described in Table 1. Gain over the test period was calculated by quadratic regression of BW on time, and DMI was equal to the total cumulative DMI over the same period. Average daily feed intake (ADFI) and ADG were calculated with the appropriate length of trial in the denominator. Average daily gain was plotted on ADFI for each group and four steers in each Cartesian quadrant that were the furthest distance from the bivariate means for ADFI and ADG were selected for a total of 16 steers per group. Table 2 illustrates breed composition by Cartesian quadrant. Animals with medical or health issues may have affected either feed intake or GN were removed from the selection group. The range of ADFI was 6.8–17.3 kg/day and for ADG the range was 1.0–2.4 kg/day.

2.3. Tissue collection and RNA isolation

Longissimus dorsi muscle, subcutaneous adipose samples were from near the tailhead, liver tissue from section VII of the right lobe, rumen papillae, the mucosal epithelial layers of rinsed duodenum, jejunum, ileum removed from the small intestine wall with a sterile glass slide, and spleen tissue were collected from 2010-born steers (discovery population) with positive and negative residual GN (n = 14) and rumen papillae and ileum mucosal samples were collected from sixteen 2011 spring-born and sixteen 2011 fall-born steers (validation population). All tissue samples were collected approximately 10–30 min post-

Table 1
Rations on a dry matter basis provided to steers *ad libitum*.

	Discovery population (n = 14)[1]	Spring validation population (n = 16)[1]	Fall validation population (n = 16)[1]
Dry rolled corn	82.7		57.35
Corn silage	12.75		
Supplement[2]	4.5		
High-moisture corn		57.75	
Wet distillers grains with solubles		30	30
Alfafa Hay		8	8
Steakmaker® with monensin		4.25	4.25
Urea			0.4

[1] Discovery population of steers was on study for 70 days, Spring validation population received *ad libitum* feed for 64 days and fall validation animals were on test for 92 days.
[2] The supplement contained (% DM) 62.55% Limestone, 2.38% NaCl, 32.63% Urea, 0.93% trace mineral mix (13% Ca, 12% Zn, 8% Mn, 10% Zn, 1.5% Cu, 0.2% I, and 0.1% Co), 0.56% (A 8,818,490 IU/kg, D 881,849 IU/kg, and E 882 IU/kg) and 0.95% Rumensin-80.

mortem, flash frozen in liquid nitrogen, and stored at −80 °C until they were further processed.

Total RNA was isolated from these tissues with TriZol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's directions with one modification: after samples were hand shaken with TriZol reagent and chloroform and incubated, the samples were centrifuged for 20 min rather than 15 min at 4 °C. Quantification of total RNA was performed with a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE). Reverse transcription of RNA (2 μg) with M-MLV (Promega, Madison, WI) was performed according to the manufacturer's protocol.

2.4. Real-time polymerase chain reaction (PCR)

Complimentary DNA (5 ng) was used in a 10 μL reaction containing 1XSYBR green master mix (Roche Molecular Biochemical, Indianapolis, IN) and 0.48 μM forward and reverse oligonucleotide primers (Table 3). All attempts were made to design oligonucleotides to bridge an intron in order to determine whether contaminated genomic DNA was present. Each oligonucleotide set was used in a separate real-time PCR reaction. Real-time reverse transcriptase PCR (RT-PCR) was performed in triplicate using LightCycler® 480 SYBR Green I PCR Master Mix (Roche Molecular Biochemical, Indianapolis, IN), 1 μL of 5 ng/μL cDNA template and 0.48 μM each primer. The PCR was performed on a LightCycler 480 Real-Time instrument (Roche) at 95 °C for 5 min one time followed by 45 cycles at 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s and a final melting curve from 65 to 97 °C. A pooled control sample for each tissue type was amplified with target primer sets on each plate. The same pooled sample was also amplified with housekeeping primer sets on all plates. Multiple housekeeping genes were evaluated for stability using the GeNorm software (Vandesompele et al., 2002). Stable housekeepers were identified using GeNorm for spleen and liver (*Cyclophillin G*) and muscle, rumen, jejunum, ileum and duodenum tissues (*Ribosomal protein 18S*). Previously, *LCORL* was shown as constitutively expressed in adipose from steers (Lindholm-Perry et al., 2013) and used as a housekeeper for adipose in the current study. The threshold cycle or crossing point (Cp) for each target gene and the appropriate housekeeping gene from each sample was determined and used to calculate the ΔΔCt using the reference pooled cDNA samples. The fold difference between samples was obtained using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Relative quantity of expression was calculated using a relative standard curve method by plotting Cp from each sample against the logarithmic values of standard amounts of pooled cDNA (Cikos et al., 2007).

Table 2
Breed composition of validation steers (n = 32).

Breed	High gain high intake	High gain low intake	Low gain low intake	Low gain high intake
Hereford	0	0.03125	0	0.09375
Angus	0.1875	0.1875	0.03125	0.15625
Maine Anjou	0.0625	0.09375	0	0
Chiangus	0	0.0625	0.03125	0
Red Angus	0	0.046875	0.09375	0.09375
Charolais	0.0625	0.15625	0.1875	0.03125
Shorthorn	0	0	0.03125	0.09375
Beefmaster	0.0625	0.1875	0	0
MarcII[1]	0	0	0.03125	0.03125
Simmental	0.09375	0.078125	0	0.15625
Brangus	0.0625	0	0.125	0.125
Bonsmara	0	0	0	0.03125
MarcIII[2]	0.0625	0.03125	0.0625	0
Limousin	0.03125	0	0	0
Salers	0.0625	0	0.09375	0
Romosinuano	0	0	0.03125	0
Santa Gertrudis	0.15625	0	0	0.0625
Brown Swiss	0	0	0.09375	0
Brahman	0	0.09375	0.09375	0
Gelbvieh	0.09375	0	0	0

[1] MarcII animals are 25% each of Simmental, Gelbvieh, Hereford, and Angus.
[2] MarcIII cattle are 25% each of Pinzgauer, Red Poll, Hereford, and Angus.

2.5. Analysis

Discovery population: Breed effects were not included in the analysis of the data from the discovery population. The objective was to identify underlying biological mechanisms associated with differences in GN in beef cattle and sampling across breeds allowed robust estimation with greater diversity in beef cattle. Expression and phenotype were analyzed with a model including high or low gain groups as fixed effects. *Validation population*: The validation population was designed as a repeated 2 × 2 factorial (high *vs.* low ADG and high *vs.* low ADFI). The model included fixed effects for ADG class, ADFI class, their interaction and group. The main effects for ADG and ADFI were tested using contrast. Nominal *P*-values are reported. *Sequence variants*: Genotypes called from whole genome and exome capture sequence (0,1, or 2 copies of the alternate allele) of influential sires were regressed on their across-breed adjusted EPD (Kuehn and Thallman, 2014) for weaning weight, yearling weight and postweaning gain. The bulls used in regression testing were a subset of 270 sequenced bulls selected for influence on the breed evaluation study (Kuehn et al., 2008). The sequenced bulls were sires or grandsires of 65% of the steers evaluated for feed intake, and the bulls used in association testing had across-breed adjusted EPD and sufficient coverage of the RHOG variants to call genotypes from sequence.

3. Results

3.1. GN phenotypes for discovery animals (n = 14)

Seven genes were examined for transcript abundance in eight tissue types from animals with positive and negative residual GN. The phenotypic and expression data is provided in Table 4. The mean average gain

Table 3
Oligonucleotide primer sequences for real-time PCR.

Gene	Gene name	Forward primer	Reverse primer	GenBank accession number	Amplicon length (bp)
LOC525033	Olfactory receptor, family 52, subfamily K, member1-like	CTCAGATGTGATCCACCACTTT	CAGGACTCTGTTGAAGATGCAG	XM_002693427	118
LOC618173	Olfactory receptor, family 52, subfamily B, member 2-like	CTAACCTCACGAGCTCGATTTT	GGTTGTTGAAGGTCAAGTCTCC	XM_002693445	106
RRM1	Ribonucleotide reductase M1	CTCTCCCACTCTCTTCAATGCT	TGTCATAAATGCCTTCAATGCT	BC109486	98
STIM1	Stromal interaction molecule 1	CACCTTCCATGGTGAGGATAAG	CATATGTGATCAGCCACTGCAC	BC151386	116
RHOG	ras homolog gene family, member G	GACTAACTCTGGCACCCCTTG	CTGGTGTTCCCAAGCAGAGTAG	BC114882	113
PGAP2	Post-GPI attachment to proteins 2	AGGAGACTACGGCCACACACT	AGTGCAGACCGATGCAGAAG	NM_001099111	114
NUP98	Nucleoporin 98 kDa, transcript variant X1	CAATGCCTTTGCACAAAATAAG	CCAAAAGGATTAGAGGTGGTGT	XM_002693428	104
Cyclophilin G[1]	Peptidylprolyl isomerase G	TGTGTGCCCCAAAACATGCGAGAA	TGGATTCCCCTCCTCGTCCATTT	NM_001109807	175
RPS18[2]	Ribosomal protein S18	GTGGTGTTGAGGAAAGCAGACA	TGATCACACGTTCCACCTCATC	NM_001033614	57
LCORL[3]	Ligand dependent nuclear receptor corepressor-like protein	GTGAACCAGAAGAGCTGACTGA	GTTCCTCTGTTGGTGTTGACTG	NM_001192357	125

[1] Bos taurus *peptidylprolyl isomerase G* (*cyclophilin G*) was used as the housekeeping gene for liver and spleen.
[2] *Ribosomal protein S18* was used as the housekeeping gene for muscle, rumen and small intestine tissues. Oligonucleotide primer sequences were obtained from Ireland et al. (2009).
[3] *LCORL* was used as the housekeeping gene for adipose (Lindholm-Perry et al., 2013).

in the low gaining group was 98.7 kg and average gain within the high gain animals was 131 kg. The expression data was obtained by averaging the raw Cp values from all 14 discovery animals.

3.2. Gene expression in animals with positive and negative residual GN

The transcript abundance of *RHOG* in the rumen papillae samples was significantly correlated with GN in the 14 discovery animals ($P = 0.02$; Table 5). *LOC618173* was also correlated with residual GN in the ileum mucosal tissue ($P < 0.05$). There were trends towards association between *STIM1* with GN in the rumen papillae ($P = 0.08$) and *LOC525033* and *PGAP2* with GN in the ileum epithelial samples ($P \leq 0.1$; Table 5).

3.3. Validation of ruminal papillae STIM1 and RHOG expression

The transcript abundance of *STIM1* and *RHOG* were examined in the rumen papillae tissue from 32 animals with divergent ADG and ADFI in attempt to validate the relationship between expression and ADG. The expression of these genes was not significantly different among these animals (Table 6).; however, like the discovery steers, the animals with greater GN tended to have greater transcript abundance of *RHOG* in the rumen papillae.

3.4. Validation of ileum LOC618173, LOC525033 and PGAP2

The transcript abundance of *LOC618173*, *LOC525033* and *PGAP2* was also examined in the mucosal ileum tissue from 32 steers with variation in ADG and ADFI. One of the olfactory receptors, *LOC525033* (*OR52K1-like*), did not amplify in 22 of the 32 animals tested; thus, we were unable to validate the expression of this gene within this population. No differences were detected for the expression of *LOC618173* (*OR52B2-like*) or *PGAP2* and ADG in this group of steers (Table 6).

3.5. Analysis of sequence variants in RHOG

An additional 25 sequence variants located within the RHOG loci that were identified by whole genome and exome capture sequencing in 121 USMARC bulls influential in the breeding scheme for the discovery and validation populations, were tested for association with GN traits including weaning weight (WW), yearling weight (YW) and post-weaning gain (PWG). Of these markers, two (rs109252863, rs109462398) located at BTA15:52073941 and BTA15:52074152 bps, respectively, were associated with WW ($P \leq 0.03$). Two additional markers (rs1190961015, rs211337252) located at BTA15:52073955 and BTA15:52077168 bps, respectively, were associated with PWG ($P \leq 0.03$).

4. Discussion

While our previous GWAS showed an association between GN and SNP within BTA15:51.79–52.03 Mb, a region that harbors the seven genes tested in this study, our goal to identify differences in transcript abundance of these genes in animals with variation in GN on a panel of tissues was only successful in a small discovery population of animals. We were unable to verify these transcript abundance differences in a larger population of animals that were divergent for both ADG and ADFI. There are many potential explanations for this, some of which

Table 4
Phenotypic data from the discovery population of high and low residual gain steers (n = 14).

Phenotype	Min[1]	Max	Mean	SD
High gain (n = 7)	131.1	150.1	138	8.1
Low gain (n = 7)	98.7	113.8	106	5.4

Tissue	*LOC525033*[2]	*LOC618173*	*RRM1*	*STIM1*	*RHOG*	*PGAP2*	*NUP98*	*LCORL*	*18S*	*CycloG*
Adipose	36.5 (2.16)	37.9 (2.28)	35.2 (2.28)	30.2 (3.44)	28.9 (1.72)	32.5 (2.57)	33.1 (4.34)	30.2 (1.59)		
Duodenum	32.9 (4.97)	28.2 (1.62)	27.8 (2.37)	25.7 (4.38)	24 (1.99)	27.7 (1.66)	25.2 (2.27)		19.4 (0.82)	
Ileum	32.7 (3.7)	30 (2.18)	29.7 (5.49)	26.8 (3.39)	25.3 (3.22)	29.2 (3.23)	29.1 (5.02)		19.9 (2.06)	
Jejunum	33.3 (3.92)	29.7 (1.85)	29.2 (3.47)	27.2 (2.44)	25.7 (2.16)	29.2 (1.69)	28.3 (2.67)		20.5 (1.62)	
Liver	31.6 (3.56)	28.7 (1.98)	24.9 (0.62)	23 (0.64)	22.9 (0.69)	25 (0.54)	22.6 (0.6)		23.4 (0.96)	
Muscle	33.5 (2.41)	30.7 (1.52)	25.6 (0.71)	22.6 (0.41)	24.4 (0.45)	27.7 (0.61)	23.1 (0.46)		20.2 (0.36)	
Rumen	32.0 (3.26)	29.4 (1.57)	26.8 (0.64)	23.8 (0.65)	22.8 (0.4)	23.52 (0.61)	23 (0.6)		16.1 (0.36)	
Spleen	29.3 (4.3)	26 (1.24)	25.2 (0.7)	23 (0.56)	20.5 (0.53)	25.8 (0.5)	22.2 (0.49)			26 (1.43)

[1] Values are presented in units of kg.
[2] Units of expression are raw Cp values averaged over the 14 discovery animals tested.

Table 5
LSMeans and standard errors for gene expression in population of steers (n = 14) with diverse residual gain phenotypes.

Gene	Phenotypic Group[1]	Adipose		Muscle		Rumen		Duodenum		Jejunum		Ileum		Liver		Spleen	
		LSMEANS (SE)[2]	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*	LSMEANS (SE)	*P*
LOC525033	High	−0.016 (0.292)	0.2	−0.34 (0.177)	0.4	−0.15 (0.191)	0.8	0.091 (0.241)	0.6	0.28 (0.221)	0.5	0.025 (0.172)	0.1	−0.92 (0.262)	0.8	0.14 (0.166)	0.3
	Low	−0.69 (0.377)		−0.13 (0.177)		−0.16 (0.191)		−0.081 (0.197)		0.073 (0.204)		−0.18 (0.172)		−0.84 (0.262)		−0.13 (0.166)	
LOC618173	High	−0.42 (0.300)	0.9	−0.25 (0.167)	0.4	−0.43 (0.288)	1.0	−0.26 (0.128)	0.3	2.03 (1.096)	0.2	**0.33 (0.188)**	**0.02**[3]	−0.60 (0.349)	0.4	1.41 (0.946)	0.1
	Low	−0.34 (0.300)		−0.042 (0.180)		−0.31 (0.311)		−0.046 (0.128)		−0.21 (1.096)		**−0.42 (0.188)**		−1.02 (0.377)		−0.72 (0.946)	
RRM1	High	0.14 (0.171)	0.3	−0.091 (0.052)	0.4	−0.062 (0.055)	1.0	0.11 (0.183)	0.4	−0.58 (0.228)	0.6	−0.12 (0.374)	1.0	−0.43 (0.127)	1.0	−0.33 (0.120)	0.5
	Low	0.42 (0.153)		−0.025 (0.05)		−0.061 (0.055)		−0.12 (0.183)		−0.41 (0.228)		−0.13 (0.404)		−0.43 (0.127)		0.097 (0.120)	
STIM1	High	−0.33 (0.155)	0.9	−0.016 (0.031)	0.5	−0.070 (0.044)	0.08	−0.00017 (0.103)	0.4	−0.18 (0.080)	0.1	−0.058 (0.162)	0.4	−0.46 (0.135)	0.8	−0.0037 (0.148)	0.5
	Low	−0.29 (0.144)		0.018 (0.031)		−0.049 (0.044)		−0.118 (0.095)		−0.0016 (0.080)		−0.27 (0.162)		−0.52 (0.1)		0.15 (0.148)	
RHOG	High	−0.19 (0.085)	0.6	−0.024 (0.030)	0.2	**0.10 (0.030)**	**0.02**[3]	0.027 (0.146)	0.3	−0.14 (0.124)	0.8	0.066 (0.150)	0.3	−0.39 (0.135)	0.5	0.050 (0.135)	0.7
	Low	−0.12 (0.085)		−0.087 (0.030)		**−0.008 (0.030)**		−0.19 (0.146)		0.082 (0.124)		−0.16 (0.150)		−0.49 (0.1)		0.12 (0.135)	
PGAP2	High	−0.22 (0.078)	0.4	0.032 (0.050)	0.4	−0.0039 (0.048)	0.7	−0.056 (0.109)	0.4	0.0075 (0.050)	0.4	0.18 (0.132)	0.09	−0.33 (0.089)	0.3	0.062 (0.149)	0.5
	Low	−0.12 (0.066)		−0.028 (0.050)		−0.029 (0.048)		−0.20 (0.109)		0.067 (0.050)		−0.17 (0.132)		−0.48 (0.089)		0.20 (0.149)	
NUP98	High	−0.042 (0.267)	1.0	−0.0053 (0.031)	0.1	0.078 (0.045)	0.2	0.031 (0.175)	0.4	−0.40 (0.160)	0.3	0.1 (0.293)	0.6	−0.29 (0.069)	0.5	−0.010 (0.130)	0.5
	Low	−0.054 (0.267)		−0.075 (0.031)		0.0009 (0.045)		−0.19 (0.175)		−0.17 (0.148)		−0.1 (0.316)		−0.36 (0.069)		0.19 (0.130)	

The bolded values are those significant at $P < 0.05$.
[1] Gain phenotypes divided into high and low categories. High gain animals are those with total gain values of over 115 kg. Low gain animals gained under 115 kg over the study.
[2] LSMEANS for high and low gain groups are reported. Standard errors for each are in parenthesis.
[3] Values in bold are significant at $P < 0.05$.

Table 6
LSMeans and standard errors for gene expression in population of steers (n = 32) with diverse feed intake and gain phenotypes in A. rumen papillae and B. ileum epithelial tissue.

A.

Phenotypic	*STIM1*[2]		*RHOG*[2]	
Group[1]	LSMEANS[3]	*P*	LSMEANS	*P*
1	0.02 (0.04)	0.6	0.07 (0.04)	0.1
2	0.02 (0.04)		0.03 (0.04)	
3	0.006 (0.04)		−0.04 (0.04)	
4	−0.05 (0.04)		0.01 (0.04)	

B.

Phenotypic	*LOC618173*[4]		*PGAP2*[4]	
Group[1]	LSMEANS	*P*	LSMEANS	*P*
1	0.11 (0.2)	0.6	0.15 (0.06)	0.5
2	−0.15 (0.2)		0.05 (0.06)	
3	0.08 (0.2)		0.05 (0.06)	
4	0.12 (0.2)		0.07 (0.06)	

[1] Phenotypic groups of steers are: 1 = High gain, high intake; 2 = High gain, low intake; 3 = Low gain, low intake; 4 = Low gain, high intake.
[2] *STIM1* and *RHOG* were evaluated for transcript abundance in the rumen tissue of steers.
[3] Values reported are LSMEANS with standard errors in parentheses.
[4] *LOC618173* and *PGAP2* were evaluated for transcript abundance in the ileum of steers.

may include: 1) a more significant association between one or more of these genes exists within a tissue type that we did not test, 2) the association between this region and GN is due to a translation or a protein alteration rather than a change in mRNA abundance, 3) the gene or genes controlling GN in the population of animals used to evaluate RNA transcripts does not reflect the same regions' influence detected in our SNP/GWAS discovery population, or 4) management differs among contemporary groups, and things such as diet modifications are influencing or masking gene expression differences. Measuring the transcript abundance of a variety of potential candidate genes located in a region detected by SNP GWAS analysis prior to fine mapping may not be the most effective way to examine genes for their role in functional phenotypic variation influence *via* mRNA differences.

A cluster of markers on the high density SNP BeadChip between 51.8 to 52.0 Mb were significantly associated with GN; with an additional marker also associated at approximately 51.4 Mb. The region between 51.4 and 51.8 is gene rich and contains a cluster of olfactory receptors and RNA polymerase II subunit pseudogenes. This study was limited to 2 olfactory receptors located closer to 51.8 Mb. We chose to limit our transcription abundance assay to two located at ~ 51.778 Mb (*LOC525033* or *OR52K1-like*) and 51.796 Mb (*LOC618173* or *OR52B2-like*) because there were several additional SNP more highly associated with GN located further downstream of this region. Thus if an olfactory receptor or RNA polymerase gene in this gene rich region is responsible for variation in GN, it is possible that we failed to it. Olfactory receptors are interesting candidates for feed efficiency as their expression has been detected in the gut and may be related to feed intake. Olfactory receptors in the gut may serve as sensor of chemical or nutritional status and may have a role in nutrient absorption or digestive function (Palouzier-Paulignan et al., 2012). A recent study by Primeaux et al. (2013) detected the up-regulation of three olfactory receptors in the duodenum of obesity prone rats fed a high fat diet for 14 days. These receptors appear to have functions in the small intestine that may affect or be affected by intake that play a role in variation of GN. We detected a potential relationship between GN and the expression of the olfactory receptor-like gene LOC618173 ($P < 0.05$); however, we were unable to validate the expression of this gene with GN in a second population of animal.

Four of the SNP in the original GWAS study were located within the *STIM1* gene loci and *STIM1* showed a trend towards association with GN in the rumen tissue of our discovery population ($P = 0.08$). A recent study has shown that STIM1 in the mouse tongue induces fatty acid

calcium signaling and may influence an animal's preference for a high fat diet because *Stim1*−/− mice lose their preference for long chain fatty acids (Dramane et al., 2012; Abdoul-Azize et al., 2013). There is no such supporting literature regarding this gene in cattle and we did not examine the tongue for *STIM1* expression; however, there is some precedence for *STIM1* expression in the stomach since expressed sequence tags for *STIM1* have been identified in both human and mouse stomach tissue. In addition, *STIM1* appears to have a growth-related function because *Stim1*−/− knockout mice are significantly smaller in size than their wild type littermates (Mancarella et al., 2013). While there could be a plausible role between this gene's function and GN in cattle, it may be important to note that the SNP from the original cattle GWAS were all located within *STIM1* introns (Snelling et al., 2011). These SNP may be in LD with the causative mutation for GN in this gene or another gene located nearby.

Our initial sample size of animals with positive and negative residual GN was small (n = 14). However, the GWAS study produced fairly significant SNP in this region, and since the animals chosen for this study were divergent for GN, a large number of animals may not necessarily be required to detect differences in expression, especially if the gene of interest is responsible for the variation in GN in a single tissue type. Indeed, we were able to detect associations between some of these genes in certain tissues and residual GN. However, a larger sampling of animals than we used to validate these effects may have been beneficial since we were only able to see a trend between GN and one of the genes (*RHOG*) detected as differentially expressed in our discovery population. This may be due, in part, to the difference in the experimental models used to select animals in the discovery and validation populations. In the discovery population feed intake was limited to ±0.32 SD of the mean and animals that were divergent in GN were selected, whereas, steers for the validation population were divergent for both feed intake and GN. In addition, our discovery population consisted of only one animal with partial *bos indicus* influence, whereas our validation population consisted of more *bos indicus* breed influence. The region on BTA15 does not appear to have been previously associated with growth traits in *bos indicus* cattle (Santana et al., 2014) and it is possible that inclusion of those breeds among our population may have masked some of the potential expression differences.

RHOG in the rumen was associated with residual GN ($P = 0.02$) in the discovery population. While only a trend existed in the validation population between this gene and ADG, *RHOG* produced similar results in both populations with the same direction of expression in both groups of animals (*i.e.*, animals with greater GN had greater levels of *RHOG* expression). RHOG is a small GTPase that functions as a molecular switch in signal transduction cascades. In general, Rho family proteins function in reorganization of the actin cytoskeleton and may regulate cell proliferation, cell shape, cell attachments, and cell motility. It is relatively highly expressed in human stomach tissue (Hs.501728) and has previously been reported as expressed in the rumen tissue of cattle (Bt.4307). In a recent study by Yang et al. (2012), RHOG was identified as part of a positive feedback loop that activates PI3K. PI3K is part of the Akt/mTOR signaling pathway, the latter of which is a "master regulator" of protein synthesis (Inoki et al., 2003). Thus, stimulated PI3K leads to increased cellular signaling for protein synthesis. Presumably, in the current experiment, the increased expression of *RHOG* in the rumen is associated with increased cellular signaling for rumen papillae protein synthesis and perhaps this improves the function of the papillae or possibly papillae number or size for nutrient or VFA adsorption, which may improve gain.

The association of four SNP with WW and PWG among 121 sires influential in the populations of animals used for this study also suggests that *RHOG* may be influencing GN traits in these populations. One of these markers (rs109462398) was a synonymous coding sequence SNP (c.216A > G; NM_001080305.1), two of the others were located within the last intron of *RHOG* (rs109252863 and rs1190961015) and the fourth (rs211337252) was located within an intron of *PGAP2*. None seem to be likely candidates for altering the expression of RHOG; however, further evaluation should be done prior to eliminating them as potential mutations influencing GN in cattle. While the SNP associated with GN traits do not appear to be candidates for altering expression, they could be in strong LD with regulatory variants outside coding regions. Sequence coverage outside coding regions is not sufficient for calling enough genotypes to test associations or estimating LD between variants in coding and adjacent non-coding sequence. Also, identification of variants that potentially regulate RHOG is hampered by a lack of annotated transcription factor binding sites, microRNA targets and other regulatory features.

We have identified two genes (*RHOG* and *LOC618173*) with differences in expression in the rumen and ileum of a crossbred population of cattle with that correlate with GN (with similar levels of feed intake). These genes reside within a region on chromosome 15 that was associated with GN in a previous GWAS study on crossbred animals that included 7 *bos taurus* breeds of cattle (Snelling et al., 2011). Although we detected a similar trend for *RHOG*, we were unable to validate the differences in *RHOG* transcript abundance in a second population of animals, possibly due to differences in phenotype, breed or contemporary groups. An additional validation among one or more larger populations of cattle with gain phenotypes that are not confounded by intake may be warranted in order to adequately assess whether these genes have a role in gain in the rumen and ileum of beef cattle.

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